

What is Claimed is:

1. A method of detecting a protein-protein interaction, comprising:
(a) providing a cell that contains a first heterologous conjugate and a second heterologous conjugate,
5 wherein said first heterologous conjugate comprises a first protein of interest conjugated to a detectable group,
and wherein said second heterologous conjugate comprises a second protein of interest conjugated to a protein that specifically binds to an internal structure within said cell; and then
10 (b) detecting the presence or absence of binding of said detectable group to said internal structure, the presence of said binding indicating that said first and second proteins of interest specifically bind to one another.
2. A method according to claim 1, wherein said detectable group is a protein,
15 and said first protein and said detectable group together comprise a fusion protein.
3. A method according to claim 1, wherein said cell contains and expresses a nucleic acid encoding said fusion protein.
- 20 4. A method according to claim 1, wherein said second heterologous conjugate is a fusion protein.
5. A method according to claim 5, wherein said cell contains and expresses a nucleic acid encoding said fusion protein.
- 25 6. A method according to claim 1, wherein said first and second proteins of interest together comprise members of a specific binding pair.
7. A method according to claim 6, further comprising the step of
30 administering a test compound to said cell prior to said detecting step,
and wherein the absence of binding of said detectable group to said internal structure indicates that said test compound inhibits the binding of said members of said specific binding pair.

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8. A method according to claim 1, further comprising the step of:
(c) repeating steps (a) and (b) a plurality of times with a library of proteins of interest, wherein one of said first and second proteins of interest is maintained the same and the other of said first and second proteins of interest is replaced with a different member of said library, so that said library is screened for proteins that specifically bind to one of said first or second proteins of interest.
9. A method according to claim 8, wherein said library is a combinatorial library.
10. A method according to claim 8, wherein said library comprises the expression product of a cDNA library.
11. A method according to claim 1, wherein said second heterologous conjugate further comprises a detectable group.
12. A method according to claim 1, wherein said cell is a eukaryotic cell.
13. A method according to claim 1, wherein said cell is a yeast, plant, or animal cell.
14. A method according to claim 1, wherein said cell is a mammalian cell.
15. A method according to claim 1, wherein said internal structure is the cell nucleus or a structure contained in the cell nucleus.
16. A method according to claim 1, wherein said internal structure is contained in the cell cytoplasm.
17. A method according to claim 1, wherein said internal structure is selected from the group consisting of plasma membrane, cytoskeleton, centromere, nucleus, mitochondria, endoplasmic reticulum, vacuoles, golgi apparatus, and chloroplasts,

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18. A method according to claim 1, wherein said internal structure is selected from the group consisting of the plasma membrane and the cortical cytoskeleton.

5 19. A method according to claim 1, wherein said protein that specifically binds to an internal structure is a translocatable protein, and wherein said method further comprises the step of inducing translocation of said second heterologous conjugate prior to said detecting step.

10 20. A method according to claim 1, wherein said first protein is a translocatable protein, and wherein said method further comprises the step of inducing translocation of said first protein prior to said detecting step.

15 21. A method according to claim 1, wherein said protein that specifically binds to an internal structure is selected from the group consisting of cytosolic protein kinases, protein phosphatases, adapter proteins, cytoskeletal proteins, cytoskeleton associated proteins, GTP-binding proteins, plasma transmembrane proteins, plasma membrane associated proteins, β -arrestin, visual arrestin, and fragments thereof that specifically bind to an internal structure.

20 22. A method according to claim 21, wherein said protein that specifically binds to an internal structure is a protein kinase C isoform or a fragment thereof that specifically binds to an internal structure.

25 23. A method according to claim 22, wherein said protein that specifically binds to an internal structure is a protein kinase C fragment selected from the group consisting of C1 domains and C2 domains.

30 24. A method according to claim 1, wherein said first and second proteins of interest are the same.

25. A method according to claim 1, wherein said first and second proteins of interest are different.

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26. A fusion protein comprising a protein that specifically binds to an internal structure within a cell and a protein that is a member of a specific binding pair.

5 27. A nucleic acid encoding a fusion protein according to claim 26.

28. A cell that contains and expresses a nucleic acid of claim 27.

10 29. A fusion protein comprising a protein that is a detectable group and a translocatable protein.

30. A nucleic acid encoding a fusion protein according to claim 29.

15 31. A cell that contains and expresses a nucleic acid according to claim 30.

32. A kit useful for detecting protein-protein interactions within a living cell, comprising:

20 (a) a cell that contains and expresses a nucleic acid encoding a first fusion protein, said fusion protein comprising a protein that specifically binds to an internal structure within said cell and a first protein of interest; together with

 (b) a vector for said cell, said vector containing an expression cassette; said expression cassette comprising a promoter operable in said cell and operatively associated with a nucleic acid encoding a detectable protein;

25 said expression cassette further comprising a splice site positioned adjacent said nucleic acid encoding a detectable protein so that a heterologous nucleic acid encoding a second protein of interest can be inserted therein to produce a nucleic acid segment encoding a second fusion protein;

 said second fusion protein comprising said detectable protein and said second protein of interest.

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33. A kit according to claim 32, wherein said vector is a plasmid.

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34. A kit useful for detecting protein-protein interactions within a living cell, comprising:

- (a) a cell that contains and expresses a nucleic acid encoding a first fusion protein, said fusion protein comprising a detectable protein and a first protein of interest; together with
- (b) a vector for said cell, said vector containing an expression cassette, said expression cassette comprising a promoter operable in said cell and operatively associated with a nucleic acid encoding a protein that specifically binds to an internal structure within said cell;
- said expression cassette further comprising a splice site positioned adjacent said nucleic acid encoding a protein that specifically binds to an internal structure within said cell so that a heterologous nucleic acid encoding a second protein of interest can be inserted therein to produce a nucleic acid segment encoding a second fusion protein,
- said second fusion protein comprising said protein that specifically binds to an internal structure within said cell and said second protein of interest.

35. A kit according to claim 34, wherein said vector is a plasmid.

36. A nucleic acid library comprising a plurality of separate nucleic acids, each of said separate nucleic acids encoding a fusion protein, said fusion protein comprising a protein of interest and a detectable protein, wherein said protein of interest encoded by each of said separate nucleic acids is different from the protein of interest encoded by the other nucleic acids of said library.

37. A nucleic acid library according to claim 36, wherein said detectable protein comprises a green fluorescent protein or a protein that specifically binds a fluorescent group.

38. A nucleic acid library according to claim 36, wherein said library is a cDNA library.

39. A nucleic acid library according to claim 36, wherein said library is a combinatorial library.

40. A nucleic acid library comprising a plurality of separate nucleic acids,
5 each of said separate nucleic acids encoding a fusion protein, said fusion protein comprising a protein of interest and a protein that specifically binds to an internal structure within a cell, wherein said protein of interest encoded by each of said separate nucleic acids is different from the protein of interest encoded by the other nucleic acids of said library.

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41. A nucleic acid library according to claim 40, wherein said protein that specifically binds to an internal structure within a cell is a translocatable protein.

42. A nucleic acid library according to claim 40, wherein said protein that
15 specifically binds to an internal structure is a protein kinase C isoform or a fragment thereof that specifically binds to an internal structure.

43. A nucleic acid library according to claim 40, wherein said protein that specifically binds to an internal structure is a protein kinase C fragment selected from
20 the group consisting of C1 domains and C2 domains.

44. A nucleic acid library according to claim 40, wherein said library is a cDNA library.

25 45. A nucleic acid library according to claim 40, wherein said library is a combinatorial library.

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